Catching evaders

Summary

This thesis deals with study of tick cysteine-rich chemokine binding proteins Evasin-3 and Evasin-4 by several biophysical techniques such as NMR spectroscopy, X-ray crystallography, and surface plasmon resonance biosensor analysis. In the **Chapter 1** known approaches of disulfide mapping in cysteine-rich peptides are reviewed. The short review of tick cysteine-rich proteins is given in the second part of this chapter.

Chapter 2 establishes a novel technique of mapping disulfide bond connectivity using selenocysteine scanning (SecScan) and NMR spectroscopy. It has been shown that single substitution of cysteine by selenocysteine causes selective perturbations of Cα and Cβ chemical shifts across the mixed S-Se bond. This approach was validated on cysteine-rich peptide and proteins with known disulfide connectivity – μ-conotoxin KIIIA, Kalata B1, and bovine pancreatic trypsin inhibitor (BPTI). SecScan was applied to unravel disulfide network of a tick saliva protein Evasin-3. Obtaining three synthetic truncated Evasin-3 variants with single Cys/Sec substitution we showed presence of inhibitory cystine knot in the structure of this protein. Applicability of SecScan has been further expanded to expressed proteins. Using *E.coli* expression system with the reprogrammed genetic code, disulfide connectivity of a tick protein BSAP1 has been established.

Chapter 3 describes a solution NMR spectroscopy and solid phase peptide synthesis approach to study the chemokine-binding tick protein Evasin-3 and its complex with CXCL8. The high-resolution structure was obtained for the Evasin-3 core region and used in molecular docking with CXCL8. We showed that Evasin-3 disrupts CXCL8 dimer and prevent its binding to GAGs. Having elucidated structure of Evasin-3, two novel CXCL8 binders were derived from the Evasin-3 sequence. Both compounds showed nM affinity to CXCL8 and effectively inhibit CXCL8-induced migration of polymorphonuclear neutrophils (PMN).

Chapter 4 reports on application of a fluorescently labelled Evasin-3 variant for imaging of CXCL1 deposited on endothelium cell wall in inflamed arteries. Solution NMR spectroscopy was used to prove that Evasin-3 binds CXCL1 but leaves one of GAG binding sites unoccupied and vacant for GAG binding. The fluorescent label was incorporated to Evasin-3 site-selectively during solid phase peptide synthesis and was shown to not affect affinity to CXCL1. Obtained fluorescently labelled Evasin-3 was successfully applied for imaging CXCL1 in human microvascular endothelial cells and on activated endothelium of mouse carotid arteries.

Chapter 5 addresses the study of Evasin-4 by NMR spectroscopy and X-ray crystallography. Recombinantly expressed Evasin-4 was crystallize and its structure resolved by X-ray crystallography. Evasin-4 crystallizes in dimeric form and retains the similar to Evasin-1 three-dimensional fold. Formation of the complex of Evasin-4 with CCL5 was followed by solution NMR spectroscopy. We showed that Evasin-4 binds N-terminal region of CCL5 disrupting CCL5 dimer. Moreover, Evasin-4 binding to CCL5 is not affected by presence of high excess of CXCL4 with which CCL5 form biologically active heterodimers.